Prostate Cancer: Serum and Tissue Markers

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The detection of prostate cancer, its clinical staging, and the prediction of its prognosis remain topics of paramount importance in clinical management. The digital rectal exam, although once the "gold standard," has been largely supplanted by a variety of techniques including serum and tissue-based assays. This article reviews recent progress in the development of prostate-specific antigen assays with greater specificity; molecular markers for prostate cancer (DNA ploidy, nuclear morphometry, markers of proliferation, and cell adhesion molecules); the link between vitamin D deficiency and the clinical emergence of prostate cancer; the possible correlation of serum insulin-like growth factor levels with the risk for developing prostate cancer; and the latest advances in radiologic staging. [Rev Urol. *2001;3(suppl 2):S11–S19*]

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The detection of prostate cancer, its clinical staging, and the prediction of its prognosis remain topics of paramount importance in clinical management. The digital rectal exam, although once the "gold standard," has been largely supplanted by a variety of techniques including serum and tissue-based assays. Methods for analysis of PSA have been improved, and a host of other tumor markers and biologic determinants are on the horizon. Advances in body imaging have also provided new capability for noninvasive assessment.

PSA: An Update

Nothing has been more helpful to advance the management of men with prostatic carcinoma than the development of reliable assays for prostate specific antigen (PSA). Although this analyte was first exploited as a tool to identify prostate cancer in tissue sections, the application with the greatest impact has been in early detection and/or screening, where it is used to evaluate risk potential for the presence of malignancy. Despite the tremendous advantages of this, the most important of all human tumor markers, PSA is not an ideal test. Problems, including lack of sensitivity and specificity and the risk of over-detection, are real. The greatest liability with PSA, according to most experts, is its relative lack of specificity. Thus, tremendous efforts have been made to make PSA more specific. Lack of specificity and the inherent false-positive rate is expensive in an economic sense, because it mandates further testing, including antibiotics, ultrasound biopsy, and pathology charges. Moreover, it is expensive emotionally: telling a man that he has an elevated PSA causes a great deal of worry for him as well as for his loved ones.

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In an effort to make PSA more specific, so-called PSA derivatives, including PSA velocity, age-specific PSA, and PSA density have been realized. Although there has been considerable enthusiasm for these approaches, none have been shown in broad-based clinical trials to be useful in early detection. Age-specific cutoffs suffer from a lack of sensitivity, PSA density and transition zone density suffer from sampling bias, and both PSA velocity and the free/total ratio suffer from test variability.

The recognition that PSA circulates in a number of molecular forms has provided significant enhancement in specificity. Most of PSA in serum is not in the free form found in the ejaculate, but rather it is complexed to protease inhibitors. These include alpha-2 macroglobulin and alpha-1 antichymotrypsin (ACT).[1–4] The majority of PSA in the circulation is complexed to ACT. Where the complex is formed has not yet been determined. Scandinavian investigators demonstrated that measurement of the free/total ratio of PSA increased the specificity of total PSA alone.[2,5] Subsequent studies[6,7] confirmed these findings. The definitive study in this regard was that reported by Catalona et al.[8] For example, in this multicenter trial, at the 95% sensitivity level, using a cutoff of 25% led to an enhancement of test specificity of 20% over that afforded by total PSA.

Several problems exist, however, when trying to determine the free/total PSA ratio. There are significant concerns over pre-analytic handling of the specimens, because free PSA is less stable than complexed PSA.[9,10] Moreover, different manufacturers' assays have given staggeringly different results. The quotient necessary for this ratio makes the bias particularly problematic.[11,12] These issues make the free/total PSA ratio less useful in broad-based clinical settings.

Because of the concerns over the practicality of the free/total PSA assay, much effort has been expended in attempting to develop specific assays for the complexed form of PSA. This approach is derived from the fact that the ACT complexed form of PSA is the analyte that is more specific for prostatic carcinoma. The Bayer Corporation developed a specific assay for complex PSA that has been shown to be highly reproducible as well as specific.[13] A study was performed on archival serum utilizing this assay in men undergoing ultrasound guided needle biopsy.[14] In comparison with the Hybritech Tandem R free and total assays, enhanced specificity was found with the Bayer complex form of PSA. For example, at the 95% sensitivity level the specificity of the total PSA was 22%, that of the free/total PSA ratio 15.6% (at a 28% cutoff), and the complexed PSA ratio with a cutoff of 2.52 afforded a 26.7% specificity. Similar results were obtained from an expanded multi-institutional study.[15] The potential benefits of utilizing complex PSA determination include the use of a single analyte on an elevated platform, which obviates assay variability issues, as well as cost efficiency when compared with the free/total PSA measurement, which requires two separate PSA determinations. Because the complexed PSA assay is equivalent to total PSA for monitoring or staging disease, it would thus appear to be the best

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possible form of PSA assay for all clinical uses.

Molecular Markers for Prostate Cancer

In addition to the well-tested clinical utility of stage, grade, and PSA in assessing prognosis and guiding management, the available array of biologic markers of prostate cancer continues to expand. Some of these serve only as "tumor markers" that can be used to indicate the presence of the disease. Others are more properly designated "biologic determinants," because they are components of the cell that actually have a role in establishing or maintaining the malignant phenotype. The role of a marker is not always clear with respect to establishing prognosis. Confusion arises when researchers attempt to extrapolate between the clinical and laboratory settings. It is also important to recognize that although we attempt to use molecular markers to assist in evaluating early-stage disease, much of the available information about these molecules comes from observations made on material from patients with advanced-stage disease, because of its more ready accessibility. Modern techniques, such as laser-capture microscopy, may overcome this deficit. In addition, the application of any marker to the clinical setting is an extremely complex issue. The reader is referred, therefore, to the criteria for evaluation of prognostic markers and reviews addressing statistical literature that have previously been published.[16,17] The array of candidates available to enhance routine measurements of grade and stage is enormous and cannot be exhaustively reviewed in this summary. For the sake of brevity, only DNA ploidy, nuclear morphometry, markers of proliferation, and cell adhesion molecules will be considered.

The DNA content of tumor cells is referred to as *DNA ploidy*. Assessment of DNA ploidy using flow cytometry and/or static imaging has been extensively investigated in prostate cancer.[18,19] Many studies have indicated that abnormal ploidy (ie, aneuploidy or tetraploidy) correlates with tumor progression, the development of metastases, and poor survival.[20,21] Whether or not DNA ploidy is an independent variable remains questionable. Assessing the impact of DNA ploidy changes in early-stage disease is complicated by the long survival times of these patients. In patients with pT2 disease, Gleason score and pre-operative PSA remain the most effective tool for predicting prognosis. However, with respect to pT3 tumors, DNA ploidy has prognostic value in predicting response to hormonal therapy.[22] Results on metastatic prostate cancer remain controversial.[23,24] Although recent attempts have been made to evaluate DNA ploidy in material from needle biopsies, it is likely that sampling errors may confound these attempts and preclude clinical utility.

With respect to pT3 tumors, DNA ploidy has prognostic value in predicting response to hormonal therapy.

In addition to the amount of DNA present in the nucleus, measurements of nuclear size and shape (termed *nuclear morphometry*) have also been reported to be of prognostic significance. In a study of T stage, Gleason score, and 10 nuclear morphometric factors (such as mean nuclear area, nuclear perimeter, shortest and longest nuclear axis, form factor, and their standard deviations), nuclear area had independent prognostic significance, but only in T1 tumors.[25] Studies on the technical aspects of these methods have suggested that the superior fixation that occurs in needle biopsy specimens may provide increased accuracy of measurement.[26] However, the widespread application of these techniques is plagued by problems with tissue handling, user dependency, standardization of automated techniques using computer equipment, quality assurance, and quality control.[27] With these limitations in mind, however, there is continuing interest in these techniques, especially with respect to surrogate endpoints for chemoprevention studies.[28]

Studies aimed at assessing the rate of proliferation in prostate carcinomas have used a variety of technical approaches. These include mitotic figure counting, static and flow cytometry, bromodeoxyuridine labeling, and immunostaining with antibodies directed against components of proliferating cells, such as proliferating cell nuclear antigen (PCNA), Ki-67, p21, and cyclin D1.[29–31] Essentially all of these studies have revealed very low rates of proliferation in most tumors. Attempts have also been made to correlate proliferative activity with disease outcome.[32,33] Although most studies have indicated strong correlation between features such as proliferation and histologic

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grade and/or tumor stage, there are only very limited data to suggest an independent prognostic significance associated with measurements of proliferation indices.

One of the hallmark features of lethal malignancies is their ability to invade and/or metastasize. Obligate to this process is the loss of intercellular adhesion that normally holds epithelial cells together at their sites of origin. Within normal tissues, epithelial cells have a variety of mechanisms that attach them to neighboring cells and to their basement membrane. Assessing the status of mechanisms by which cells adhere is probably important for both providing biologic insight as well as developing prognostic markers. Some studies have suggested that an inverse relation exists between the down-regulation of selected cell adhesion molecules in high-grade prostatic intraepithelial neoplasia (HGPIN) and prostate cancer and the up-regulation of invasion-related enzymes believed to be involved in the degradation of basement membrane, such as collagenases and matrix metalloproteinases.[34,35] The adhesion molecules that have attracted the most attention are the integrins, the cadherins, C-CAM, and CD44. The integrins are a large family of heterodimeric plasma membrane receptors composed of alpha and beta subunits. The patterns and combinations of subunits that are expressed appear to be different in normal epithelium, HGPIN, and carcinoma. In general, loss of expression is associated with the malignant phenotype and tumor progression.[36,37] Specifically, losses of integrins α2, α4, α5, αv and β4 have been reported. Higher expression of α6 is correlated with increased invasion.[38] The β1C variant, reported to inhibit tumor growth, is down-regulated in prostate cancer.[39] E-cadherin is a well-known member of another large family of proteins associated with calcium-dependent adhesion. Several investigators have indicated that a correlation exists between reduction or loss of E-cadherin expression and advanced-stage, higher-grade prostate cancer. Although one third of organ-confined prostate cancers exhibit abnormal E-cadherin expression, more than 75% of metastatic carcinomas have this abnormality.[40] C-CAM is a calcium-independent molecule that actually belongs to the immunoglobulin family of genes. Its sequence is also similar to carcinoembryonic antigen. Decreased expression has been reported in HGPIN, and its expression was not detected in carcinomas.[41] This suggests that C-CAM is a possible suppressor gene that is altered early in prostatic oncogenesis. CD44 is another transmembrane protein believed to be involved with cell–cell and cell–matrix interactions. A soluble form of CD44 known as variant 5 (v5) was found to be lower in the serum of patients with prostate cancer or benign prostatic hyperplasia (BPH) than controls.[42] However, down-regulation of the CD44 standard isoform has been reported to occur in the majority of prostate cancers.[43] Decreased expression was also found to correlate with high-grade and DNA aneuploidy.[44] To the contrary, other authors have reported that increased CD44 expression correlates with high grade and advanced stage. Clearly, additional studies are necessary to resolve these differences.

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Vitamin D and Prostate Cancer: From Laboratory to Bedside

Although it is recognized that prostatic cancer is an androgen-dependent disease, it is now very apparent that a variety of other steroid and peptide hormones are capable of regulating the growth and differentiation of prostatic cancer cells. Early epidemiologic studies have indicated that the clinical emergence of prostate cancer may be at least partially explained by vitamin D deficiency.[45] In addition to age and race, these studies linked geographic location to prostate cancer mortality through potential actions on vitamin D levels. The link between latitude and vitamin D action was clear, because ultraviolet light is required for the initial conversion of the vitamin D precursor 7-dehydrocholesterol to vitamin D3. What remained to be shown was that there was a biologic basis for the remainder of this hypothesis.

Vitamin D is known to act through both nuclear receptors (the genomic pathway) and cell membrane receptors (the non-genomic pathway). Vitamin D receptors had been found in various non-prostate benign and malignant epithelial cells. Using nuclear receptor binding assays,

Miller et al were the first to show that specific, high-affinity binding sites for the active metabolite of vitamin D (1α,25-dihydroxyvitamin D3) are present in prostate cancer cells.[46] The number of nuclear vitamin D receptors per cell varies widely among different cell lines ranging from approximately 500 to 18,000.[47,48]

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Recently, it has been found that many of the prostate cancer cell lines that were used for such studies actually represent contaminants from PC-3. This finding has led to the interesting observation that ALVA-31 cells, which is now known to be a subline of PC-3, have much higher expression of vitamin D receptors than the parental line. The mechanisms through which this increased expression has occurred are not presently known. If such a change could be induced, however, this could prove to be a valuable therapeutic approach.

It is known that vitamin D can have a marked antiproliferative effect of various non-prostate malignant cell lines in vitro. This has also been found in various prostatic carcinoma cell lines. However, it is interesting to note that the degree of antiproliferative effect varies widely between cell lines. This suggests that whereas some cells remain sensitive to the effects of vitamin D, others may have become resistant. The mechanisms through which cells become vitamin D resistant are also currently under study and may provide critical insights into the use of vitamin D in the therapy of prostate cancer.

Treatment of patients with high doses of vitamin D would likely be stymied by the hypercalcemic effects of this compound. To date, however, hypercalcemic effects in treated prostate cancer patients have been relatively inconsequential.[45] In addition, numerous synthetic vitamin D analogues have been developed that have increased pro-differentiative effects while displaying a lower hypercalcemic potential. Studies at the University of Colorado in prostate cancer cells have indicated that those analogues with hexafluorinated side chains and increased saturation of the bonds in this chain have a greater antiproliferative effect on prostate cancer cells than does the parent molecule.[49] Clinical trials with these compounds are attractive but are currently not possible owing to lack of appropriate agents approved for use in humans.

Studies with vitamin D and its analogues have also made it clear that effects on differentiation accompany the antiproliferative effects. Using the well-characterized prostate cancer cell line LNCaP, University of Colorado researchers have found that vitamin D and its analogues increase the synthesis and release of both PSA and prostate-specific acid phosphatase into the culture medium. This finding is of great clinical potential, because it indicates that in clinical trials, an early increase in a patient's serum PSA levels might indicate success rather than failure.

The mechanisms through which vitamin D induces a decrease in proliferation are now becoming clear. Preliminary results indicate that an increase in the cell cycle mediator p21 appears to be necessary for the antiproliferative effects. This increase occurs within days and could be caused by indirect actions on other genes. Consistent with this possibility, it has also been found that in some cells, but not others, p21 induction by vitamin D appears to require a prior induction of transforming growth factor beta. Again, the mechanisms of this induction, and alternate pathways will probably offer clues to methods for the appropriate use of these compounds in the management of clinical disease.

Finally, it has been found in studies on prediagnostic sera levels that decreased levels of vitamin D metabolites accompany both the presence of prostate cancer and its biologic aggression. More interestingly, it is now known that as many as 37% of hospitalized patients whose vitamin D intake is greater than the current recommended daily allowance can be vitamin D deficient. It also appears that vitamin D deficiency may be particularly common among men with hormone-refractory or advanced-stage prostate cancer. It would seem reasonable, therefore, to include vitamin D supplementation into treatment strategies for these patients. To this end, the ability of vitamin D to potentiate the effects of chemotherapeutic agents, not otherwise found to be of great value in the treatment of prostate cancer, has been examined. Vitamin D can have a synergistic effect on growth arrest of prostate cancer cells induced by cisplatin or carboplatin.[50] These findings have led to a clinical trial, in which patients are supplemented with vitamin D prior to receiving carboplatin therapy. Patients are also given dexamethasone prior to either of these agents. The results are preliminary but indicate that as many as 70% of men will respond to such therapy with decreases in their PSA of more than 50% over prolonged periods of time.

These findings indicate the potential for vitamin D and perhaps other forms of differentiation therapy to contribute to our armamentarium for the treatment of prostate cancer.

The Insulin-Like Growth Factor System in the Control of Carcinoma of the Prostate

The insulin-like growth factor (IGF) system is a complex group of molecules composed of three ligands (insulin, IGF-I, and IGF-II) and seven known binding proteins that can either enhance or inhibit the effects of the ligands.[51] The system has been studied in carcinomas of the breast, sarcomas, Wilms' tumors, and colorectal cancers.[52] Early studies on the role of this system in prostate cancer demonstrated that levels of binding protein 2 were increased at the same time that levels of binding protein 3 were decreased in the serum of patients with advanced-stage disease.[53] At the tissue level, it was next shown that the expression of binding protein 2 mRNA and protein were increased in carcinomas.[54] However, although the levels of binding proteins 3 and 4 were decreased, the levels of their mRNA expression had remained constant, suggesting the regulation of their protein levels was post-translational.[54] This observation is important, because protein 2 contains an RGD sequence and can increase cell motility, whereas binding proteins 3 and 4 are growth inhibitory.[54] An increase in protein 2 accompanied by decreases in proteins 3 and 4 could cause cells to proliferate and become more metastatic.[54]

The next advance in these studies came through the development of a series of cell lines derived from benign prostatic epithelial cells infected with SV40 large T antigen. A series of cell lines, including p-69, M-2182, and M-12 were established by passage as xenografts.[55] Although p-69 is poorly tumorigenic and nonmetastatic, the M-12 line is 100% tumorigenic and metastatic when implanted either orthotopically or intraperitoneally. Transfections were used to create an IGF type-I receptor over-expressing cell, called M-12 LISN, and its receptor-deficient counterpart, M-12 LNL-6 cells. Radioligand binding and Scatchard analysis revealed that the LISN cells contained significantly more receptors per cell than the LNL-6 cells. Soft agar assays for anchorage-independent growth revealed that LISN cells formed 75% fewer colonies than LNL-6 cells, suggesting that expression of receptors should decrease the tumorigenicity of these cells. Consistent with this postulate, the volumes of tumors formed in xenografts with LISN cells were 6-fold less than those derived from LNL-6 cells. p-69 cells were also found to contain very small amounts of telomerase activity, whereas M-12 cells contained approximately 100-fold higher levels. However, when the transfectants were examined, LISN cells were found to contain 10-fold less telomerase than LNL-6 cells. This suggests that the LISN cells are more terminally differentiated but at the same time more responsive to growth factors. The results of these studies could form the basis for a specific approach to gene therapy, in which either the expression of the IGF receptors or the expression of IGFs themselves are modified.

With respect to human applications of this research, six attempts have been made to correlate serum IGF-I levels with the risk for developing prostate cancer.[56] Three of these have shown no association, and three have shown association. The interesting aspect of this conundrum is that different assays were used to obtain the results between the different trials. It would seem that until there is better standardization of assays and larger numbers of patients can be examined, we will not be able to accurately assess the true relations of this pathway. However, studies by Wolk et al[57] on IGFBP-3 and IGF-I levels in the serum of prostate cancer patients may provide some additional insight. These workers found that in patients who had serum PSA levels of 3 or less, the presence of an elevated level of IGF-I and lower levels of IGFBP-3 were indicative of the presence of cancer. Thus, although it is not yet clear that the IGF axis is useful to diagnose cancer by itself, it may be of assistance in clarifying otherwise ambiguous serum PSA results.

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What's New in Radiologic Staging?

Much of our approach to clinical management of prostate cancer patients is highly dependent on accurate clinical staging. Although a number of surrogate markers, such as serum PSA levels and Gleason scores, have helped in assessing and predicting stage, they are not always as accurate as we would desire. Ultimately, the preferred approach to staging would be an actual visualization of the whereabouts of locally invasive or metastatic prostate cancer, such that the stage of patients could be objectively defined rather than predicted.[58,59]

At present, radionuclide bone scans remain the most effective way

to screen the entire skeleton for metastases in high-risk patients. Once abnormalities are detected, additional evaluation may be necessary using computed tomography (CT) or magnetic resonance imaging (MRI). In addition, conventional x-rays can often be used to confirm the nature of suspicious areas from bone scans. CT and MRI can also be used to evaluate patients for abdominal or pelvic lymphadenopathy. The relatively limited sensitivity of these studies has led to evaluation of MRI spectroscopy, indium-111 capromab pendetide (ProstaScint), and positron emission tomography (PET) for this purpose. At present, however, the use of these alternate approaches remains experimental.

In patients with a high clinical suspicion of extraprostatic disease and no evidence of distant metastases, MRI remains a reasonable approach to evaluate for adenopathy or local extension of carcinoma.

The use of color and/or power Doppler ultrasound may increase the sensitivity of detection of primary lesions in the prostate, but reported results have been variable.[60–62] Ultrasound contrast agents are currently under investigation. Initial studies with these agents suggest that contrast agents improve sonographic accuracy both in detection and in determining the extent of local invasion.[63] Other technical advances including harmonic imaging and three-dimensional ultrasound are also being evaluated.

The use of radiolabeled antibodies directed against proteins on the surfaces of malignant cells has long been investigated as a method to detect small quantities of those cells anywhere in the body. Several different antibodies have been used to evaluate prostatic carcinomas. At present, ProstaScint is approved for clinical use and a relatively large experience with it has accumulated.[64–69] Because of limited experience in controlled trials, however, its precise role in clinical management is not yet well defined. Situations in which some practitioners find it of clinical utility include: 1) evaluation of residual or recurrent disease after local treatment including prostatectomy; and 2) providing prognostic information or facilitating treatment planning at the time of initial diagnosis. However, many find the limited spatial resolution in these images as well as the high background associated with this agent to be unacceptable.

MRI, especially using an endorectal coil, is the most accurate cross-sectional imaging method available to evaluate the local extent of prostate carcinoma invasion.[70–76] Because of its cost and limitations, however, it cannot be universally recommended. In patients with a high clinical suspicion of extraprostatic disease and no evidence of distant metastases, MRI remains a reasonable approach to evaluate for adenopathy or local extension of carcinoma.

PET imaging offers the potential to detect malignant cells based on their increased glucose metabolism wherever they are located within the body.[77–82] The potential exists to use PET in the pretreatment search for metastases to the bone or lymph nodes. This technique could also be applied to the detection of recurrences after various forms of local treatment. PET appears to be very useful for determining the extent of disease with several nonprostatic malignancies. However, some preliminary studies in patients with prostate cancer have been disappointing. Its use at present should, therefore, remain investigational.

One additional imaging modality should also be considered. Proton magnetic resonance spectroscopy (MR spectroscopy) can be used to evaluate tissue metabolic activity as reflected by local concentrations of metabolic breakdown products.[83–85] One group of investigators has found that spectroscopy added to conventional rectal coil MRI improves the accuracy in determining both the intraprostatic location and extent of involvement by carcinoma.[86] Evidence has also been presented indicating that MR spectroscopy can improve accuracy in the diagnosis of extracapsular disease. At present, however, the technique remains largely experimental and is not widely available. ■

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Main Points

- Much effort has been expended in attempting to develop specific assays for the complexed form of PSA; one company has developed a specific assay for complex PSA that has been shown to be highly reproducible as well as specific.
- Because the complexed PSA assay is equivalent to total PSA for monitoring or staging disease, it would thus appear to be the best possible form of PSA assay for all clinical uses.
- Many studies have indicated that abnormal DNA content in tumor cells (DNA ploidy) correlates with tumor progression, the development of metastases, and poor survival; whether or not DNA ploidy is an independent variable remains questionable.
- Some studies have suggested that an inverse relation exists between the down-regulation of selected cell adhesion molecules (eg, the integrins, the cadherins, C-CAM, and CD44) in prostate cancer and the up-regulation of invasion-related enzymes believed to be involved in the degradation of basement membrane.
- Early epidemiologic studies have indicated that the clinical emergence of prostate cancer may be at least partially explained by vitamin D deficiency.
- Although it is not yet clear that the insulin-like growth factor axis is useful to diagnose cancer by itself, it may be of assistance in clarifying otherwise ambiguous serum PSA results.
- At present, radionuclide bone scans remain the most effective way to screen the entire skeleton for metastases in high-risk patients; once abnormalities are detected, additional evaluation may be necessary using CT or MRI.
- PET imaging offers the potential to detect malignant cells based on their increased glucose metabolism wherever they are located within the body; however, some preliminary studies in patients with prostate cancer have been disappointing. Use of PET at present should, therefore, remain investigational.

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